Supplementary Appendix

Intensity-modulated radiation therapy (IMRT) planning protocol in this trial

All patients were immobilized in the supine position using a thermoplastic mask that covered the head, neck, and shoulder regions. Both nonenhanced CT (for dose calculation) and contrast-enhanced CT (for target delineation) images were obtained from the vertex to 2 cm below the stemoclavicular joint at a 3 mm slice thickness.

The gross tumor volume (GTV), clinical target volume (CTV), cervical lymph node tumor volume (GTVnd) and organs at risk (OAR) were contoured slice by slice on computed tomography images. The GTV encompassed the extent of the tumors defined by the computed tomography/MRI imaging studies. Only one CTV was delineated, which included the GTV plus a 0.5-1.0 cm margin. The planning target volume (PTV) was delineated, including PTVnx, PTVnd, and PTV1, which were the external expansions of GTVnx, GTVnd, and CTV by a certain distance, generally 0.3 cm in the forward, up, down, left, and right directions and 0.1~0.3 cm in the backward direction. The OARs surrounding the CTV included the brainstem, spinal cord, optic nerves, optic chiasm, pituitary gland, lens, temporal lobes, parotid glands, temporomandibular joints, mandible, etc.

The prescribed doses were 60 Gy, 60 Gy and 54 Gy in 27 fractions for the PTVs derived from GTVnx, GTVnd and CTV, respectively. The threshold of doses to the OARs for reirradiation of recurrent NPC is listed in the **Protocol**[1].

Genomic DNA preparation and next-generation sequencing

Genomic DNA from frozen tissues and FFPE samples was extracted using the

DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) and QIAamp DNA FFPE Tissue Kit (Qiagen, Germany), respectively, following the manufacturer's protocol. Degradation and contamination were monitored on a 1% agarose gel, and the concentration was measured by using the Qubit® DNA Assay Kit in a Qubit® 2.0 fluorometer (Life Technologies, CA, USA).

For whole-exome sequencing (WES), qualified genomic DNA from tumors and matched peripheral blood from 18 rNPC patients was fragmented by Covaris with resultant library fragments of 180-280 bp, and then adapters were ligated to both ends of the fragments. Extracted DNA was then amplified by ligation-mediated PCR (LM-PCR), purified, and hybridized to the Agilent SureSelect Human Exome V6 for enrichment; nonhybridized fragments were subsequently washed out. Both uncaptured and captured LM-PCR products were subjected to real-time PCR to estimate the magnitude of enrichment. Each captured library was then pooled and sequenced on a Illumina HiSeq X platform with 150-bp paired-end reads

Sequence data quality control

The original fluorescence image files obtained from the HiSeq platform were transformed to short reads (raw data) by base calling and recorded in FASTQ format, which contained sequence information and corresponding sequencing quality information. After excluding reads containing adapter contamination and low-quality/unrecognizable nucleotides, clean data were used for downstream bioinformatics analyses. At the same time, the number of total reads, sequencing

error rate, percentage of reads with average quality >20 and with average quality >30, and GC content distribution were calculated.

Read mapping and somatic alteration detection

Valid sequencing data were mapped to the human reference genome (UCSC hg38) by Burrows-Wheeler Aligner (BWA) software to obtain the original mapping results stored in BAM format. Then, Samblaster and Sambamba were used to sort BAM files and perform duplicate marking to generate a final BAM file for computing the sequence coverage and depth.

To call somatic single nucleotide variations (SNVs) and small insertions and deletions (InDels) from paired tumor-normal samples, MuTect and Strelka were used, respectively[2,3]. Subsequently, the VCF (variant call format) file was annotated by ANNOVAR[4].

Copy number burden (chromosomal instability)

Chromosomal instability was estimated by the Weighted Genome Integrity Index (wGII) score[5], which was computed as follows: the ploidy of the sample was first determined as the weighted median integer copy number, with weights equal to the lengths of the copy number segments. For each of the 22 autosomal chromosomes, the percentage of gained and lost genomic material was calculated relative to the ploidy of the sample. The wGII score of a sample was defined as the average of this percentage value over the 22 autosomal chromosomes.

Neoantigen prediction

To identify neoantigens, we used the NetMHC, NetMHCpan to predict neoepitopes from 8mer to 11mer. Neoepitopes with a binding affinity < 500 nM and less than wild-peptide were predicted as neoantigens..

MSI calling

MSIsensor was used to estimate the mircrosatellite instability (MSI) status for each patient. For each sample, we note the total number of sites with sufficient data (at least 20 spanning reads in both normal and tumor) and the number of somatic sites. The percentage of somatic sites is the MSI score.

References

1. Han F, Zhao C, Huang SM, et al. Long-term outcomes and prognostic factors of re-irradiation for locally recurrent nasopharyngeal carcinoma using intensity-modulated radiotherapy. *Clin Oncol (R Coll Radiol)* 2012; **24**(8): 569-76.

2. Cibulskis K, Lawrence MS, Carter SL, et al. Sensitive detection of somatic point

mutations in impure and heterogeneous cancer samples. *Nat Biotechnol* 2013; **31**(3): 213-9.

3. Saunders CT, Wong WS, Swamy S, Becq J, Murray LJ, Cheetham RK. Strelka: accurate somatic small-variant calling from sequenced tumor-normal sample pairs. *Bioinformatics* 2012; **28**(14): 1811-7.

4. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res* 2010; **38**(16): e164.

5. Niu B, Ye K, Zhang Q, et al. MSIsensor: microsatellite instability detection using paired tumor-normal sequence data. *Bioinformatics* 2014; **30**(7): 1015-6.

Table S1. Treatment exposure to toripalimab combined with intensity-modulated radiotherapy

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	Number (%) or time (days)
Toripalimab	
7 cycles	22 (88.0%)
6 cycles	2 (8.0%)
5 cycles	1 (4.0%)
Patients who received IMRT, no. (%)	25 (100.0%)
Patient who completed definitive IMRT, no. (%)	25 (100.0%)
Median (IQR) dose of IMRT (Gy)	60.21 (60 - 60.21)
Median (IQR) dose per fraction (Gy)	2.23 (2.22-2.23)
Median (IQR) duration of IMRT (days)	36 (35-38)

Data are n(%) unless otherwise specified.

Table S2. Dosimetric data (average and range) of 25 patients who completed radiotherapy.

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	rT2-3 (N = 18)	**rT4 (N = 7)**
GTV Volume (cc)	**35.0 (19.5 – 50.7)**	**52.0 (41.0 – 93.9)**
GTV D_{95} (in BED)	**75.8 (75.5 – 76.5)**	**75.4 (74.9 – 76.0)**
GTV D_{50} (in BED)	**77.9 (77.4 – 78.8)**	**77.8 (77.5 – 78.1)**
GTV D_{min} (in BED)	**73.8 (72.6 – 74.9)**	**73.0 (71.1 – 73.9)**
GTV D_{max} (in BED)	**80.3 (79.6 – 81.6)**	**81.5 (81.0 – 82.9)**

Data are median (IQR) unless otherwise specified. BED – biologic effective dose (calculated at α/β = 10 Gy; BED = D (1+ d/(α/β)), where D = total dose and d = fractional dose.

Table S3. Association among PD-L1 expression, clinical characteristics and treatment activity.

		No. of pts	Response		P value
			CR	PR+SD	
PD-L1 expression	**Positive**	**15**	13 (86.7%)	2 (13.3%)	**0.178**
	Negative	**4**	2 (50.0%)	2 (50.0%)	
T stage	**T4**	**7**	4 (57.1%)	3 (42.9%)	**0.298**
	T2/3	**18**	15 (83.3%)	3 (16.7%)	
N stage	**N1**	**9**	7 (77.8%)	2 (22.2%)	**1.000**
	N0	**16**	12 (75.0%)	4 (25.0%)	
Sex	**Male**	**18**	15 (83.3%)	3 (16.7%)	**0.298**
	Female	**7**	4 (57.1%)	3 (42.9%)	

Table S4. Hazard ratios for selected prognostic factors of progression-free survival in patients who completed radiotherapy.

	Hazard ratio (95% CI)	P value
PD-L1 positive (Yes vs No)	0.43 (0.04 - 4.75)	0.488
Age (per year increase)	1.02 (0.91 - 1.14)	0.780
Sex (female vs male)	0.44 (0.06 - 3.14)	0.413
rT category (rT4 vs rT2-3)	3.07 (0.50 - 18.84)	0.226
rN category (rN1 vs rN0)	1.65 (0.25 - 10.75)	0.602
EBV DNA copy number/mL (⩾ 1500 vs <1500)	0.81 (0.08 – 7.88)	0.859
GTV (per cc increase)	1.04 (1.01 - 1.07)	0.021
GTV D_{95} (in BED) (per Gy increase)	0.40 (0.07 - 2.01)	0.275
GTV D_{50} (in BED) (per Gy increase)	0.47 (0.11 - 1.98)	0.306
GTV D_{min} (in BED) (per Gy increase)	0.94 (0.59 - 1.52)	0.809
Tumor mutation burden (≥30% percent vs <30% percent)	2.91 (0.18 - 46.68)	0.451
Neoantigen burden (per burden increase)	0.70 (0.29 - 1.71)	0.436
Frameshift indels (per indel increase)	1.35 (0.73 - 2.50)	0.334
Copy number burden (wGII) (≥30% percent vs <30% percent)	2.78 (1.04 – 6.88)	0.037
MSI score	0.64 (0.06 - 6.39)	0.702

(≥30% percent vs <30% percent)		

Abbreviations: PFS – progression-free survival, GTV – gross tumor volume, BED – biologic effective dose (calculated at $\alpha/\beta = 10$ Gy; $BED = D(1 + d/(\alpha/\beta))$, where D = total dose and d = fractional dose.

Figure S1. Part of medical imaging evaluated as complete response.

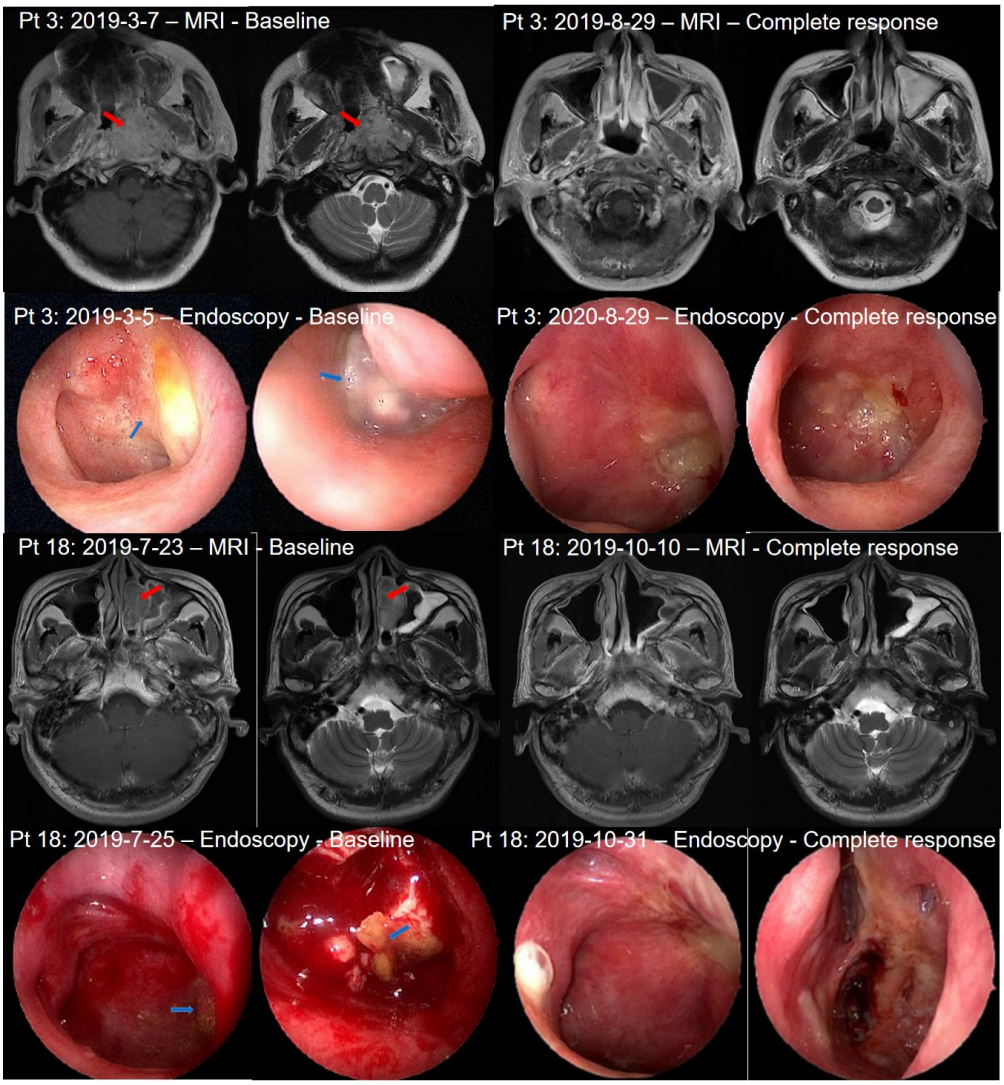

Figure S2. Association between genome characteristics and treatment activity.

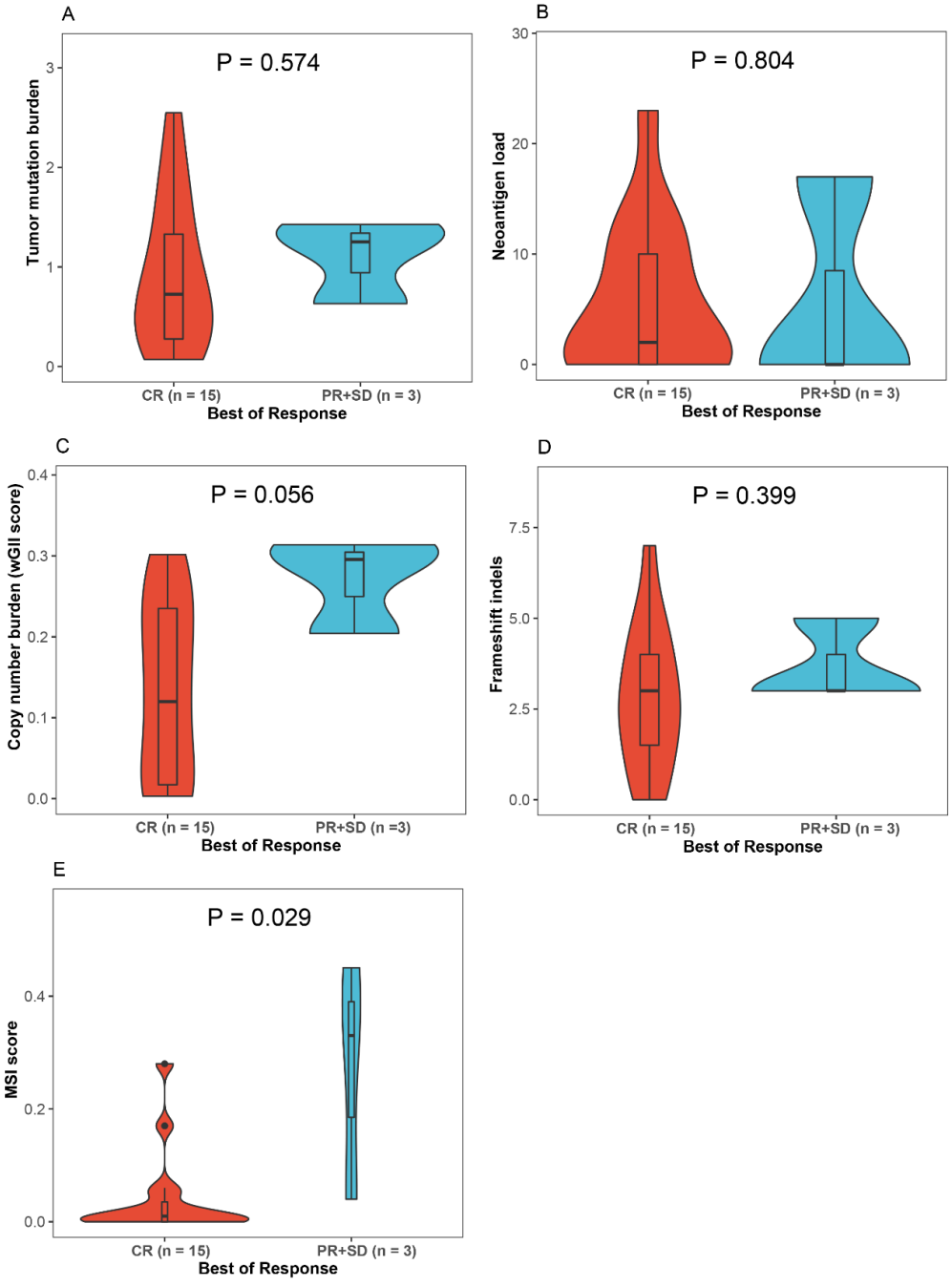

Figure S3. Genetic alterations and frequencies identified by whole exome sequencing (WES) from 18 available patients. Patients were grouped by clinical responses and progression.

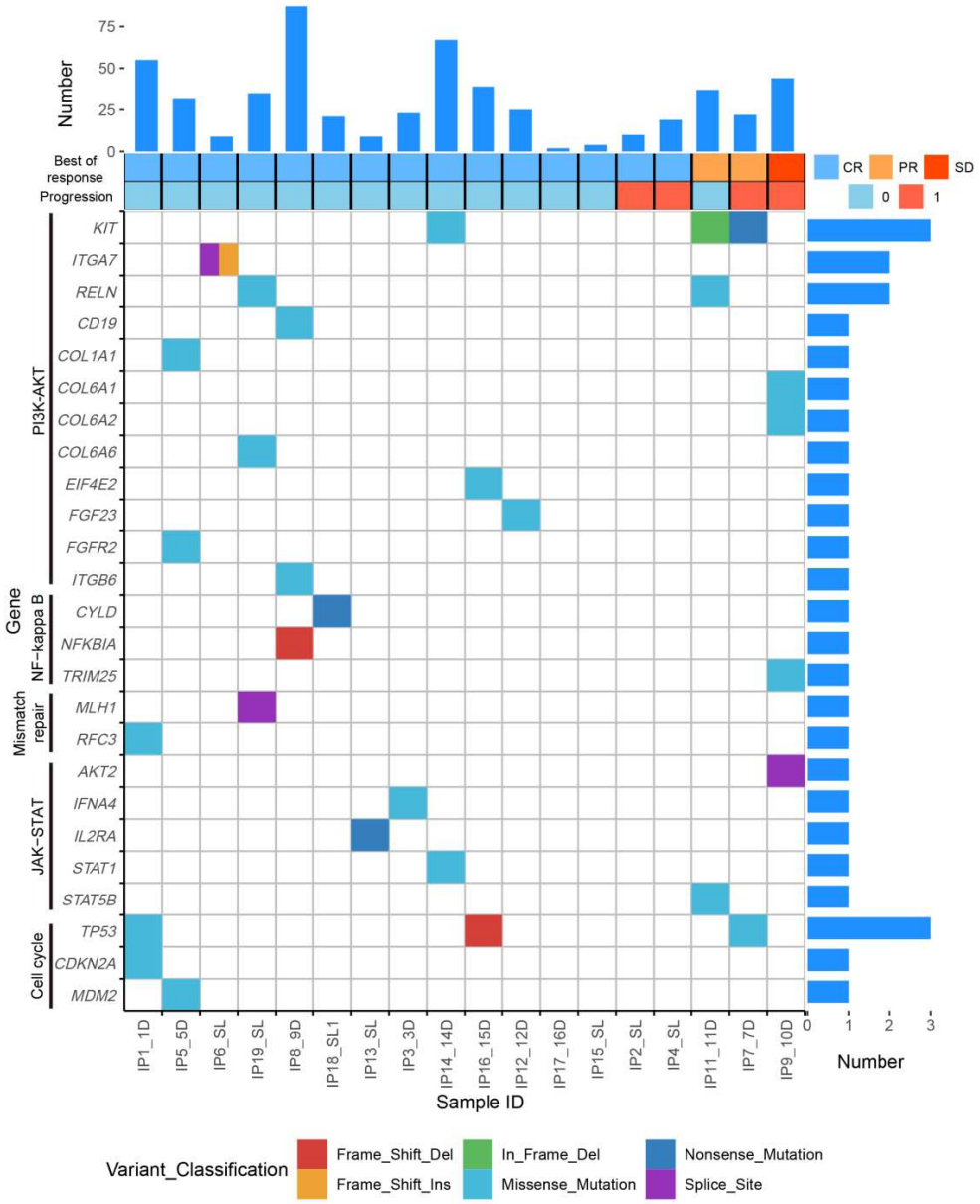

Figure S4. Association between clinical characteristics and treatment activity.